compartments. Such a mechanism has another advantage by providing flexibility. If telomere binding is switched off (e.g., by shortening the binding time), this will allow the nucleus to undergo architectural changes.

[1] I. Bronstein, Y. Israel, E. Kepten, S. Mai, Y. Shav-Tal, E. Barkai and Y. Garini, Transient anomalous diffusion of telomeres in the nucleus of mammalian cells. Physical Review Letters **103**, 018102 (2009).

1114-Plat

Mapping Neuronal Connectivity Using Stochastic Optical Reconstruction Microscopy (Storm): The Brainstorm Project

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The human brain is a highly sophisticated circuit consisting of hundreds of billions of neurons that are interconnected by an even larger number of synapses. This dense network of neurons and their connections holds key information to understanding normal brain function and perhaps what underlies its disorders. Obtaining a physical map of the brain's connectivity, however, is highly challenging due to the small size and high density of neuronal processes within a given volume. Therefore, in order to generate a map of neuronal connectivity a technique that can provide high spatial resolution and molecular specificity is needed.

We are using 3D multi-color stochastic optical reconstruction microscopy (STORM) in order to trace neuronal networks in culture at high spatial resolution. In order to capture an entire network of connections, we are using an automated, motorized piezo stage to image large areas in x-y ($\geq$ 120x120 μm) as well as in z ($\geq$ 2 μm). With this technique we can not only outline neuronal morphology at 30 μm lateral and 50 μm axial resolution, but we can also image synaptic content with high molecular specificity and identify synaptic connections. These techniques will be greatly useful for generating connectional maps of neurons in the mammalian brain and help obtain a physical understanding behind brain function.

1115-Plat

Monitoring the [ATP]/[ADP] Ratio in Beta-Cells During Glucose Stimulated Insulin Secretion Using the Genetically Encoded Fluorescent Reporter Perceval

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Pancreatic beta-cells secrete insulin in response to elevated blood glucose levels. Glucose stimulated insulin secretion depends on glucose metabolism that produces ATP. The resulting increase in [ATP]/[ADP] ratio closes ATP-sensitive potassium (KATP) channels, which leads to membrane depolarization and opening of voltage-dependent Ca2+ channels. This causes an elevation of intracellular free Ca2+ and insulin exocytosis. Insulin is secreted in a pulsatile manner, which is thought to be regulated in part by oscillations in glucose metabolism. Such metabolic oscillations would also lead to oscillations in the [ATP]/[ADP] ratio and hence regulate KATP channel activity.

Oscillations in [ATP]/[ADP] ratio have been demonstrated using biochemical and luciferase assays, but neither approach allows measurements of such oscillations in single cells. Perceval is a recently developed fluorescent protein biosensor for [ATP]/[ADP] ratio, and it permits direct measurement of [ATP]/[ADP] ratios inside living cells. We use Perceval in combination with quantitative confocal and two-photon excitation microscopy for direct measurement of the [ATP]/[ADP] ratio in beta-cells during glucose stimulated insulin secretion. For this purpose we have developed an adenoviral vector to express Perceval specifically in the beta-cells of intact mouse islets. Dynamic changes in [ATP]/[ADP] ratio can be correlated with glucose metabolism (by simultaneous imaging of Perceval fluorescence and NAD(P)H autofluorescence) and with intracellular free Ca2+ levels (by simultaneous imaging of Perceval fluorescence and the calcium sensor, FuraRed). This data allows us to test hypotheses regarding the role of localized subcellular signaling complexes and putative microdomains of glucose metabolism, [ATP]/[ADP] ratio, and Ca2+ dynamics in the regulation of glucose stimulated insulin secretion.

1116-Plat

Multiple Components Mapping of Live Tissue by Phasor Analysis of Fluorescence Lifetime Imaging

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In fluorescence lifetime microscopy (FLIM) of live tissues a major issue is the assignment of autofluorescence to specific molecular components and their interactions within the physiological context. Here we use the phasor approach to fluorescence lifetime imaging to analyze complex decays in a live tissue. The tissues used were seminiferous tubules from the testes of wild type mice or mice expressing GFP from an Oct4 transgene. Lifetime images were acquired in the time domain and analytically transformed in the phasor representation. By examination of the clustering of the phasors we identified different molecular components: auto fluorescence, GFP, collagen and retinol. Each chemical species was identified and categorized by its specific location in the phasor plot. This phasor fingerprint reduces the importance of knowing the exact lifetime distribution of the fluorophores and emphasizes the contribution of the species to the signal. To better identify specific tissue components we also used spectral imaging and second harmonic generation microscopy. Linear combinations in the same pixel of molecular species were recognized and their relative fraction was calculated and mapped. The analysis of the fluorescence decay with higher harmonics of the phasor plot separates different molecular components that have the same location in the phasor plot at one harmonic but arise from different lifetime distributions. The phasor approach to lifetime imaging in live tissue provides a unique and straightforward method for interpreting complex decays in terms of molecular features by identifying fluorophores and obtaining functional maps of their relative concentration. This method has the potential to become a non invasive tool to characterize the local microenvironment and monitor differentiation and diseases in label-free live tissues. Work supported by NIH-P41 P41-RRO3155 and P50-GM076516, NIH RO1 HD49488, NIH PO1 HD47675, CIRM RC1-00110 PD.

1117-Plat

Ultra-High Resolution Imaging of the Dynamic Nature of Post-Synaptic Molecules

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The spatial and temporal regulation in the composition of the postsynaptic membrane of synapses participates in the different forms of synaptic plasticity that trigger the cellular processes of memory formation, consolidation, and retrieval. Neurotransmitter receptors move rapidly in and out of synapses by lateral diffusion. This mobility is crucial to control the number of receptors present at a given synapse. Thus, the equilibrium between the synaptic and extra synaptic AMPA receptor number is crucial in controlling basal transmission and synaptic plasticity. This balance is regulated by the subunit composition of these receptors and by the interaction of intracellular scaffold proteins. However, how the trafficking of receptors and the scaffolding molecules in and out synapse is controlled remains unknown. Here we attempt to determine the relative distribution and trafficking properties of AMPA receptors and various scaffold proteins at unprecedented spatial ($<$ 40nm) and temporal resolution ($>$50 Hz) using a variety of novel ultra-high resolution fluorescence imaging approaches. We combine Single Particle Tracking (SPT) and Photo Activation Localization Microscopy (PALM) to map trajectories at the level of individual molecules. Here we describe the implementation of a multimodal microscope along with the development of a new dedicated analysis for single molecule segmentation and tracking. Furthermore we will discuss the application of SPT-PALM experiments on living neurons. With this novel approach, we expect to comprehend the motilities of receptors or scaffolding proteins when they traffic between the submicron sized molecular zones of dendritic spines. The combination of this type of detection and analysis will provide the information from thousands of discrete trajectories from a single cell with which it would be possible to appreciate finer details of versatile molecular mechanisms pertinent in the functioning of an excitatory synapse.

1118-Plat

Optical Recording of Electrical Activity of Cortical Layer 2/3 Pyramidal Neurons Using A Genetically-Encoded Voltage Probe

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Voltage-Sensitive Fluorescent Protein 2.3, VSFP2.3, is a genetically-encoded probe of membrane voltage using fluorescence resonance energy transfer (FRET) between a pair of cyan (CFP) and yellow (YFP) fluorescent proteins to convert voltage-activated motions of a voltage sensor domain from *Ciona intestinalis* voltage-sensitive phosphatase (Ci-VSP) into a differential voltage dependent fluorescence signal. To evaluate the utility of VSFP2.3 as a probe of electrical activity of neurons in intact brain tissue, we performed targeted whole cell current clamp and simultaneous optical recordings from L2/3 pyramidal